

Rat Nedd4

Figure1

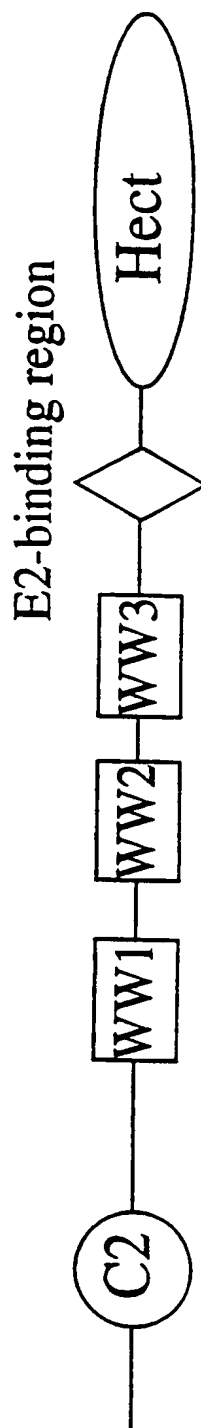


Figure 2: Clone 7.7 is the homolog of human clone

KIAA0313

- Clone 7.7 exhibits 75% identity and 95% similarity of human clone KIAA0313.

GGKDVSAEAESSMVPVTTTEAKVPMPAHIAVTPSTTKGLIARKEGRYREPPPTPPGYV
GGKDVSI EAESSLSVTTEETKVPMPAHIAVASSTTKGLIARKEGRYREPPPTPPGYI

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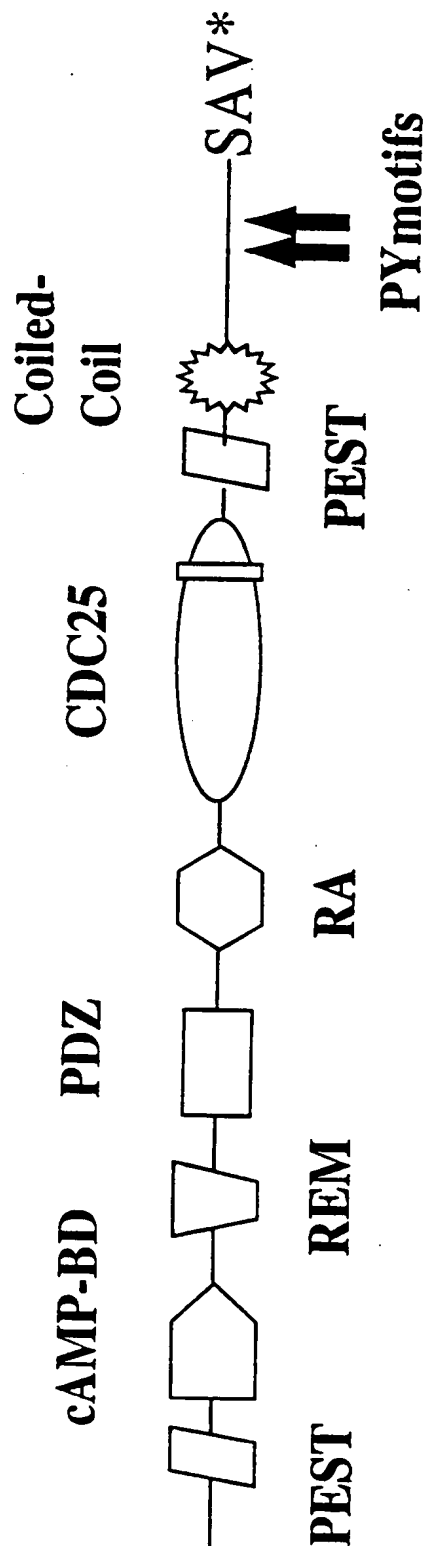
 GIPIITDFEGHSHPARKPPDYNVALQSRMVARSDDTAGPSVQQPHGHPTSSRPVNKPQ

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*****:*****
WHKPSDADPRLAPFQAGFAGAEDEDEQVSAV
WHKPNESDRLAPYQSQGFSTEDEDEQVSAV
*****:*****

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Figure 3A Schematic Diagram of GRF4



4618 b.p.

1/1 31/11
 atg aaa cca cta gca atc cca gct aac cat gga gtt atg ggc cag cag gag aaa cac tca
 M K P L A I P A N H G V M G Q Q E K H S
 61/21 91/31
 ctt cct gca gat ttc aca aaa ctg cat ctt act gac agt ctc cac cca cag gtg acc cac
 L P A D F T K L H L T D S L H P Q V T H
 121/41 151/51
 gtt tct tct agc cat tca gga tgt agt atc act agt gat tct ggg agc agc agt ctt tct
 V S S S H S G C S I T S D S G S S S L S
 181/61 211/71
 gat atc tac cag gcc aca gaa agc gag gct ggt gat atg gac ctg agt ggg ttg cca gaa
 D I Y Q A T E S E A G D M D L S G L P E
 241/81 271/91
 aca gca gtg gat tcc gaa gac gac gat gaa gaa gac att gag aga gca tca gat cct
 T A V D S E D D D D E E D I E R A S D P
 301/101 331/111
 ctg atg agc agg gac att gtg aga gac tgc cta gag aag gac cca att gac cgg aca gat
 L M S R D I V R D C L E K D P I D R T D
 361/121 391/131
 gat gac att gaa caa ctc ttg gaa ttt atg cac cag ttg cct gct ttt gcc aat atg aca
 D D I E Q L L E F M H Q L P A F A N M T
 421/141 451/151
 atg tca gtg agg cga gaa ctc tgt gct gtg atg gtg ttc gca gtg gtg gaa aga gca ggg
 M S V R R E L C A V M V F A V V E R A G
 481/161 511/171
 acc ata gtg tta aat gat ggt gaa gag ctg gac tcc tgg tca gtg att ctc aat gga tct
 T I V L N D G E E L D S W S V I L N G S
 541/181 571/191
 gtg gaa gtg act tat cca gat gga aaa gca gaa ata ctg tgc atg gga aat agt ttt ggt
 V E V T Y P D G K A E I L C M G N S F G
 601/201 631/211
 gtc tct cct acc atg gac aaa gaa tac atg aaa gga gtg atg aga aca aag gtg gat gac
 V S P T M D K E Y M K G V M R T K V D D
 661/221 691/231
 tgc cag ttt gtc tgc ata gcc cag caa gat tac tgc cgt att ctc aat caa gta gaa aag
 C Q F V C I A Q Q D Y C R I L N Q V E K
 721/241 751/251
 aac atg caa aaa gtt gaa gag gaa gga gag att gtt atg gtg aaa gaa cac cga gaa ctt
 N M Q K V E E E G E I V M V K E H R E L
 781/261 811/271
 gat cga act gga aca aga aag gga cac att gtc atc aag ggt acc tca gaa agg tta aca
 D R T G T R K G H I V I K G T S E R L T
 841/281 871/291
 atg cat ttg gtg gaa gag cat tca gta gta gat cca aca ttc ata gaa gac ttt ctg ttg
 M H L V E E H S V V D P T F I E D F L L
 901/301 931/311
 acc tat agg act ttt ctt tct agc cca atg gaa gtg ggc aaa aag tta ttg gag tgg ttt
 T Y R T F L S S P M E V G K K L L E W F
 961/321 991/331
 aat gac cag agc ctc agg gat aag gtt aca cgg gta gta tta ttg tgg gta aat aat cac
 N D P S L R D K V T R V V L L W V N N H
 1021/341 1051/351
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 F N D F E G D P A M T R F L E E F E N N

cNMP-BD

REM

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Figure 3B

4/34

1081/361

1111/371

ctg gaa aga gag aaa atg ggt gga cac cta agg ctg ttg aat atc gcg tgt gct gct aaa
L E R E K M G G H L R L L N I A C A A K

1141/381

1171/391

gca aaa aga aga ttg atg acg tta aca aaa cca tcc cga gaa gct cct ttg cct ttt atc
A K R R L M T L T K P S R E A P L P F I

1201/401

1231/411

tta ctt gga ggc tct gag aag gga ttt gga atc ttt gtt gac agt gta gat tca ggt agc
L L G G S E K G F G I F V D S V D S G S

1261/421

1291/431

aaa gca act gaa gca ggc ttg aaa cgg ggg gat cag ata tta gaa gta aat ggc caa aac
K A T E A G L K R G D O I L E V N G Q N

1321/441

1351/451

ttt gaa aac att cag ctg tca aaa gct atg gaa att ctt aga aat aac aca cat tta tct
F E N I O L S K A M E I L R N N T H L S

1381/461

1411/471

atc act gtg aaa acc aat tta ttt gta ttt aaa gaa ctt cta aca aga ttg tca gaa gag
I T V K T N L F V F K E L L T R L S E E

1441/481

1471/491

aaa aga aat ggt gcc ccc cac ctt cct aaa att ggt gac att aaa aag gcc agt cgc tac
K R N G A P H L P K I G D I K K A S R Y

1501/501

1531/511

tcc att cca gat ctt gct gta gat gta gaa cag gtg ata gga ctt gaa aaa gtg aac aaa
S I P D L A V D V E Q V I G L E K V N K

1561/521

1591/531

aaa agt aaa gcc aac act gtg gga gga agg aac aag ctg aaa aag ata ctc gac aag act
K S K A N T V G G R N K L K K I L D K T

1621/541

1651/551

cgg atc agt atc ttg cca cag aaa cca tac aat gat att ggg att ggt cag tct caa gat
R I S I L P Q K P Y N D I G I G Q S Q D

1681/561

1711/571

gac agc ata gta gga tta agg cag aca aag cac atc cca act gca ttg cct gtc agt gga
D S I V G L R Q T K H I P T A L P V S G

1741/581

1771/591

acc tta tca tcc agt aat cct gat tta ttg cag tca cat cat cgc att tta gac ttc agt
T L S S S N P D L L Q S H H R I L D F S

1801/601

1831/611

gct act cct gac ttg cca gat caa gtg cta agg gtt ttt aag gct gat cag caa agc cgc
A T P D L P D Q V L R V F K A D O Q S R

1861/621

1891/631

tac atc atg atc agt aag gac act aca gca aag gaa gtg gtc att cag gct atc agg gag
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1921/641

1951/651

ttt gct gtt act gcc acc ccg gat caa tat tca cta tgt gag gtc tct gtc aca cct gag
F A V T A T P D O Y S L C E V S V T P E

1981/661

2011/671

gga gta atc aaa caa aga aga ctt cca gat cag ctt tcc aaa ctt gca gac aga ata caa
G V I K O R R L P D O L S K L A D R I O

2041/681

2071/691

ctg agt gga agg tat tat ctg aaa aac aac atg gaa aca gaa act ctt tgt tca gat gaa
L S G R Y Y L K N N M E T E T L C S D E

2101/701

2131/711

gat gct cag gag ttg ttg aga gag agt caa att tcc ctc ctt cag ctc agc act gtg gaa
D A Q E L L R E S Q I S L L O L S T V E

2161/721

2191/731

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V A T O L S M R N F E L F R N I E P T E

PDZ

RA

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5/34

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E E V I N O E T F W V A S E I L R E T N
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C K N F N S M F A I I S G L N L A P V A
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 2521/841 2551/851
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Q D L F D P S R N M A K Y R N V L N S Q
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N L O P P I I P L F P V I K K D L T F L
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H E G N D S K V D G L V N F E K L R M I
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A K E I R H V G R M A S V N M D P A L M
 2761/921 2791/931
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F R T R K K K W R S L G S L S Q G S T N
 2821/941 2851/951
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A T V L D V A Q T G G H K K R V R R S S
 2881/961 2911/971
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 2941/981 2971/991
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L S N L E L E M D E E S L O T L S L O C
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 3061/1021 3091/1031
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T S P V A P R A G S Q Q K A Q S L P Q P
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Q Q Q P P P A H K I N Q G L Q V P A V S
 3181/1061 3211/1071
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L Y P S R K K V P V K D L P P F G I N S
 3241/1081 3271/1091
 cca caa gct tta aaa aaa att ctt tct ttg tct gaa gaa gga agt ttg gaa cgt cac aag
P Q A L K K I L S L S E E G S L E R H K
 3301/1101 3331/1111
 aaa cag gct gaa gat aca ata tca aat gca tct tgg cag ctt tct tct cct cct act tct
K Q A E D T I S N A S S Q L S S P P T S

CDC25

Insertion
Unique to
GRF4

Continuation of the
CDC25 domain

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3361/1121 3391/1131
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 P Q S S P R K G Y T L A P S G T V D N F
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 tca gat tct ggt cac agt gaa att tct tca cga tcc agt att gtt agc aat tct tct ttt
 S D S G H S E I S S R S S I V S N S S F
 3481/1161 3511/1171
 gac tca gtg cca gtc tca ctg cac gat gag agg cgc cag agg cat tct gtc agc atc gtg
 D S V P V S L H D E R R Q R H S V S I V
 3541/1181 3571/1191
 gaa aca aac cta ggg atg ggc agg atg gag agg cgg acc atg att gaa cct gat cag tat
 E T N L G M G R M E R R T M I E P D Q Y
 3601/1201 3631/1211
 agc ttg ggg tcc tat gca cca atg tcc gag ggc cga ggc tta tat gct aca gct aca gta
 S L G S Y A P M S E G R G L Y A T A T V
 3661/1221 3691/1231
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 I S S P S T E E L S Q D Q G D R A S L D
 3721/1241 3751/1251
 gct gct gac agt ggc cgt ggg agc tgg acg tca tgc tca agt ggc tcc cat gat aat ata
 A A D S G R G S W T S C S S G S H D N I
 3781/1261 3811/1271
 cag acg atc cag cac cag aga agc tgg gag act ctt cca ttc ggg cat act cac ttt gat
 Q T I Q H Q R S W E T L P F G H T H F D
 3841/1281 3871/1291
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 Y S G D P A G L W A S S S H M D Q I M F
 3901/1301 3931/1311
 tct gat cat agc aca aag tat aac agg caa aat caa agt aga gag agc ctt gaa caa gcc
 S D H S T K Y N R Q N Q S R E S L E Q A
 3961/1321 3991/1331
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 Q S R A S W A S S T G Y W G E D S E G D
 4021/1341 4051/1351
 aca ggc aca ata aag cgg agg ggt gga aag gat gtt tcc att gaa gcc gaa agc agt agc
 T G T I K R R G G K D V S I E A E S S S
 4081/1361 4111/1371
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 L T S V T T E E T K P V P M P A H I A V
 4141/1381 4171/1391
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 A S S T T K G L I A R K E G R Y R E P P
 4201/1401 4231/1411
 ccc acc cct ccc ggc tac att gga att ccc att act gac ttt cca gaa ggg cac tcc cat
 P T P P G Y I G I P I T D F P E G H S H
 4261/1421 4291/1431
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 P A R K P P D Y N V A L Q R S R M V A R
 4321/1441 4351/1451
 tcc tcc gac aca gct ggg cct tca tcc gta cag cag cca cat ggg cat ccc acc agc agc
 S S D T A G P S S V Q Q P H G H P T S S
 4381/1461 4411/1471
 agg cct gtg aac aaa cct cag tgg cat aaa ccg aac gag tct gac ccg cgc ctc gcc cct
 R P V N K P Q W H K P N E S D P R L A P

PY motifs

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SQISLLQLSTVEVATQLSMRNFELFRNIEPTEYIDDLF---KLRSKTSKANLKRFEVIN
SNVHFLHLNAYELAIQLTLQDFANFROIESTEYVDLF---ELRSRYGVPMLSKFAELVN
SAEGLDVS AKDLAQLT DHDWSLFNSIHQVELIHYVLGPQHLDVT-TANLERFMRRFN
KAECFETLSAMELAEQITLLDHIVFRSIPYE EFLQGQW--MKLDKNERTPYIMKTSQHFN
DEITLLTHPELARQTLTLEFEMYKNVKPSSELVGSPWT--KKDKVEKSPNLLKIMKHTT
VSLLEDHLEPEELSEHLTYLEFKFSFRISFDYQNYLVN----SCVKENPTMERSIALCN

: : . :: :: : . : :

QETFWVASEILRETNQLKRMKI I K H F I K I A L H C R E C K N F N S M F A I I S G L N L A P V A R L R T T
R E M F W V V S E I C A E H N I V R R M K I V K Q F I K I A R H C K E C R N F N S M F A I V S G L G H G A V S R L R Q T
E L Q Y W V A T E L C L C P V P G P R A Q L L R K F I K L A A H L K E Q K N L N S F F A V M F G L S N S A I S R L A H T
E M S N L V A S Q I M N Y A D I S S R P N A I E K W V A V A D I C R C L H N Y N G V L E I T S A L N R S P I Y R L K K T
N V T R W I E K S I T A E N Y E E R L A I M Q R A I E V M V M L E L N N F N G I L S I V A A M G T A S V Y R L R W T
G I S Q W V Q L M V L S R P T P Q L R A E V F I K F I Q V A Q K L H Q L O N F T M L A V I G G L C H S S I S R L K E T
: : * . : : : . * * . : . : : *

[illegible]

DSKVDG--LVNFEKLRLMIAKEIRHVGGRMASVNMDPALMFRTKRKK---WRSLGSLSQGS
DTRVGD--LINFELRLMLAKEVRLLTHMCSSPYDLLSILELKQSPSNALFSLNQMSASQ
HTLVEN--LINFELMRMMARAARMLHHCRSHNPVPLSPLRSRVSHLHEDSQVARISTCSE
PNFTEEG-LVNFSKMIRMISHIIREIRQFOQTAYRIDQQP-----
PDLLANTELINFSKRRKVAEIIIGEIQOYONQPYCLNEES-----
PDYLEDG-KVNVHKLLALYNHISELVQLQEVAAPPLEANKDL-----

* * * : : *

TNATVLDVAQTGG---HKKRVRRSSFLNAKKLYEDAQMARKVKQYLSNLELEMDDEESLQ
 SNAAAGTVIAANAGQATIKRRKKSTAAPNPKKMFEEAQMVRRVKAYLNSLKILSDEDLH
 -----QSLSTRSPASTWAYVQQLKVIDNQRELS
 -----KVIQYLLDKALVIDEDSLY
 -----TIRQFFEQLDPFNGLSDKQMSDYLY
 -----VHLLTSLDLYYTEDEIY

TLSLQCEPATNT
KFSLECEPAHGS
RLSRELEP----
ELSLKIEPRLPA
NESLRIEPRGCK
ELSYAREPRNHR
* * *

FIG. 4C" SEPT 1999

Figure 5: GRF4-REM domain

CDC25	-IRGGTKEALIEHLT-SHELVDAAFNVMTLITFRSILT-TREFFYALIYRY-
Sos_mouse_	-IKGGTVVVKLIERLT-YHMYADPNF-VRTFLTYRSFCK-PQELNLLIERFE
RasGEF_aimless_	VVKFASLNKLVVEHLT-HDSKHDLOFLKTFMLTYQSFT-PEKLSKLQORY-
GRF2_mouse_	-IRYASVEALLERLT-DLRFSLIDFLNTFLHTYRIFTT-ATVVLAKLSDIY-
GRF4	-IKG-TSERLTMHVVEHVVDPPTFIEDFLTYRTFLSSPMEVGKLLLEWFN
	:: : * : * . . * : * : * : * : * : *

FIG. 6A

Figure 6A:

Overall structure comparison between GRF4 and other known mammalian

RasGEF/RasGRF

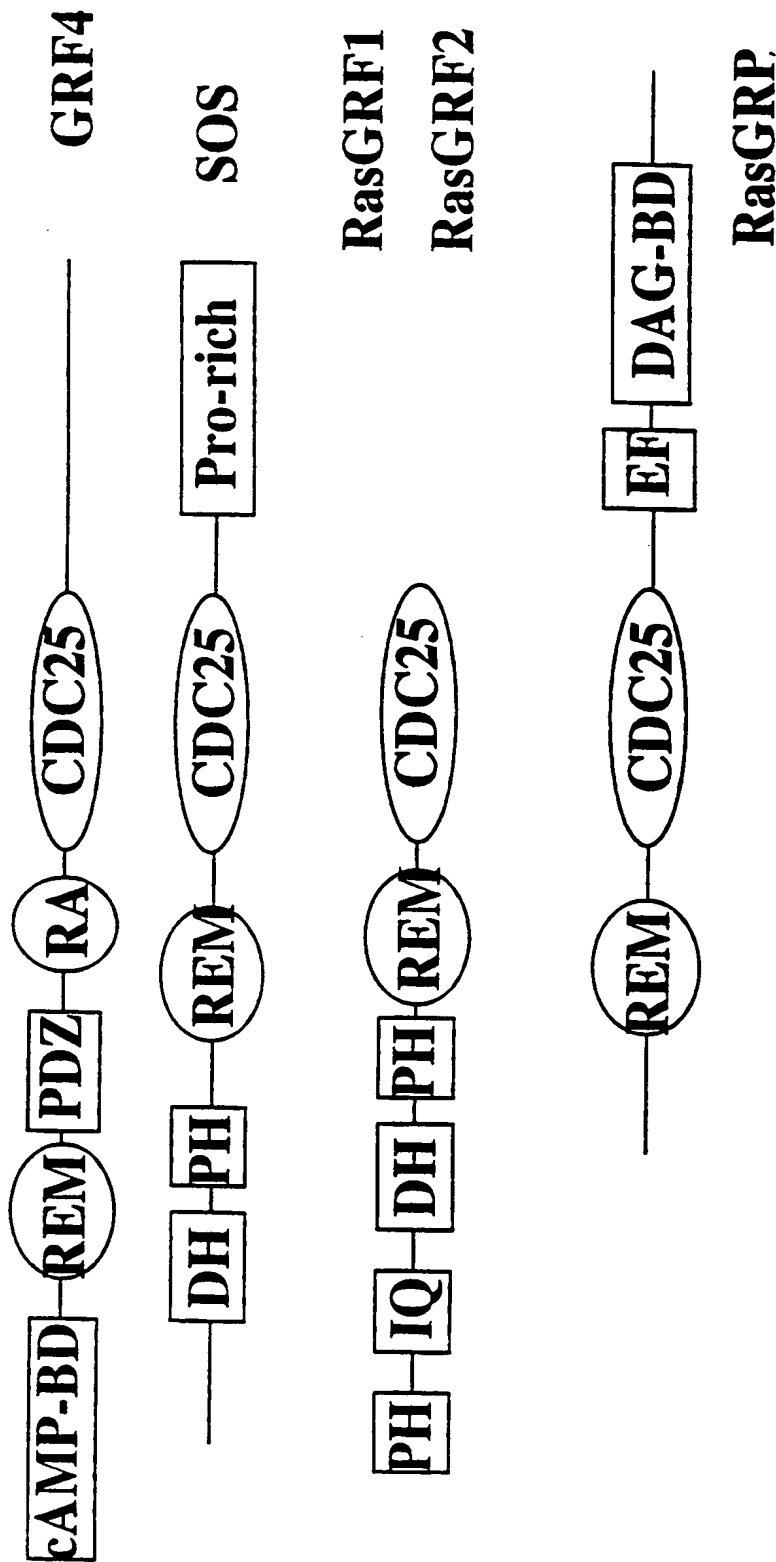
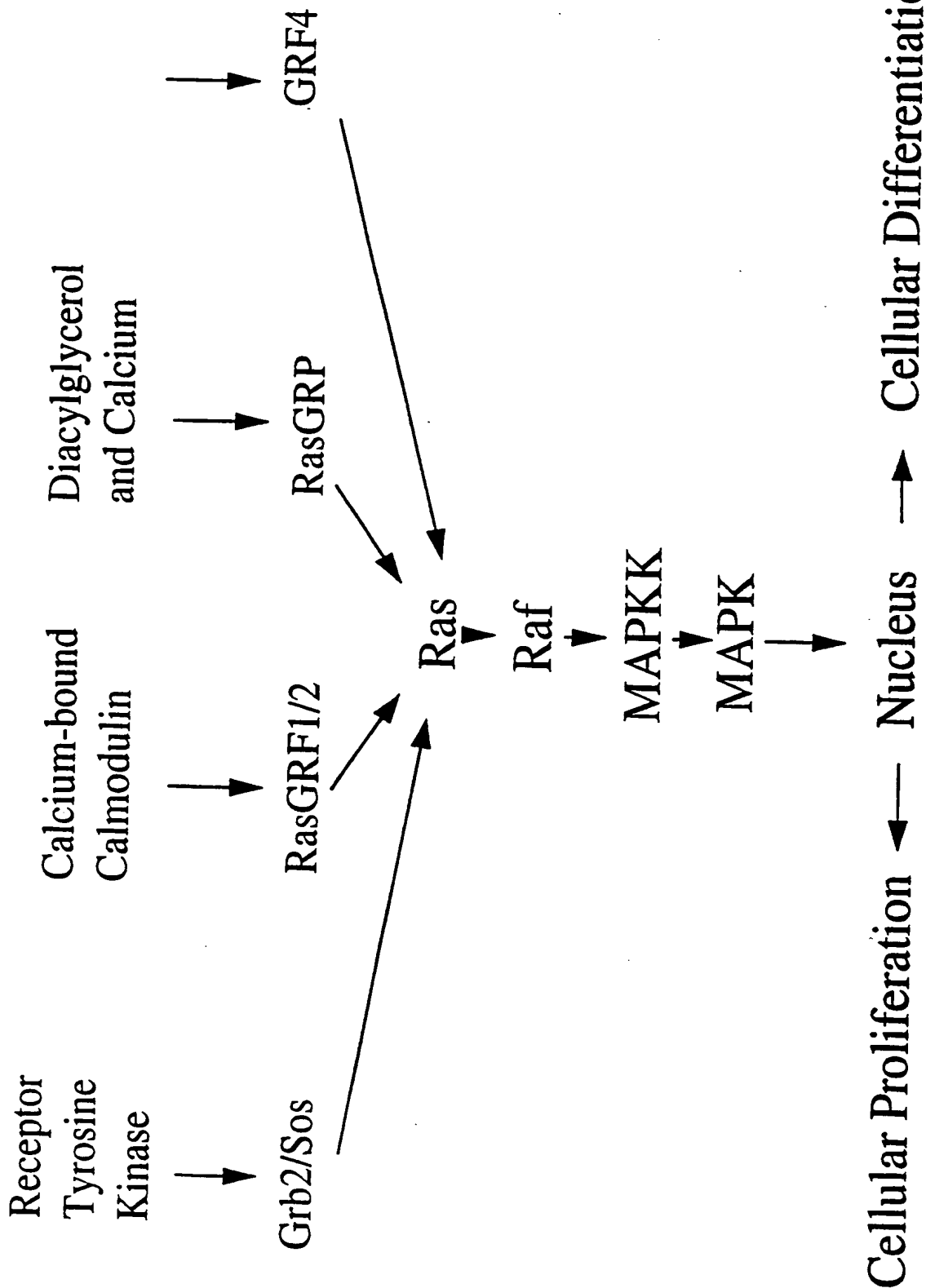


FIG. 6B

Ras Signaling pathway



1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100	2101	2102	2103	2104	2105	2106	2107	2108	2109	2110	2111	2112	2113	2114	2115	2116	2117	2118	2119	2120	2121	2122	2123	2124	2125	2126	2127	2128	2129	2130	2131	2132	2133	2134	2135	2136	2137	2138	2139	2140	2141	2142	2143	2144	2145	2146	2147	2148	2149	2150	2151	2152	2153	2154	2155	2156	2157	2158	2159	2160	2161	2162	2163	2164	2165	2166	2167	2168	2169	2170	2171	2172	2173	2174	2175	2176	2177	2178	2179	2180	2181	2182	2183	2184	2185	2186	2187	2188	2189	2190	2191	2192	2193	2194	2195	2196	2197	2198	2199	2200	2201	2202	2203	2204	2205	2206	2207	2208	2209	2210	2211	2212	2213	2214	2215	2216	2217	2218	2219	2220	2221	2222	2223	2224	2225	2226	2227	2228	2229	2230	2231	2232	2233	2234	2235	2236	2237	2238	2239	2240	2241	2242	2243	2244	2245	2246	2247	2248	2249	2250	2251	2252	2253	2254	2255	2256	2257	2258	2259	2260	2261	2262	2263	2264	2265	2266	2267	2268	2269	2270	2271	2272	2273	2274	2275	2276	2277	2278	2279	2280	2281	2282	2283	2284	2285	2286	2287	2288	2289	2290	2291	2292	2293	2294	2295	2296	2297	2298	2299	2300	2301	2302	2303	2304	2305	2306	2307	2308	2309	2310	2311	2312	2313	2314	2315	2316	2317	2318	2319	2320	2321	2322	2323	2324	2325	2326	2327	2328	2329	2330	2331	2332	2333	2334	2335	2336	2337	2338	2339	2340	2341	2342	2343	2344	2345	2346	2347	2348	2349	2350	2351	2352	2353	2354	2355	2356	2357	2358	2359	2360	2361	2362	2363	2364	2365	2366	2367	2368	2369	2370	2371	2372	2373	2374	2375	2376	2377	2378	2379	2380	2381	2382	2383	2384	2385	2386	2387	2388	2389	2390	2391	2392	2393	2394	2395	2396	2397	2398</
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Figure 7

PDZ domain

hGRF4
dGRF4
hPTP-BAS-1
hPSD-95
rLin-7-C
hdGL

hGRF4	EVNGQNFENIQLSKAMEILR
dGRF4	EVNGQSLDHVTSKRALEILT
hPTP-BAS-1	SVNSVSLEGVSHHAAIEILQ
hPSD-95	SVNGVDLRNASHEQAIALK
rLin-7-C	SVNGVSVEGEHHEKAVELLK
hdGL	EFNGINLRSATEQQARLIIG
 *
	: :

1910	1911	1912	1913	1914	1915	1916	1917	1918	1919	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938	1939	1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100	2101	2102	2103	2104	2105	2106	2107	2108	2109	2110	2111	2112	2113	2114	2115	2116	2117	2118	2119	2120	2121	2122	2123	2124	2125	2126	2127	2128	2129	2130	2131	2132	2133	2134	2135	2136	2137	2138	2139	2140	2141	2142	2143	2144	2145	2146	2147	2148	2149	2150	2151	2152	2153	2154	2155	2156	2157	2158	2159	2160	2161	2162	2163	2164	2165	2166	2167	2168	2169	2170	2171	2172	2173	2174	2175	2176	2177	2178	2179	2180	2181	2182	2183	2184	2185	2186	2187	2188	2189	2190	2191	2192	2193	2194	2195	2196	2197	2198	2199	2200	2201	2202	2203	2204	2205	2206	2207	2208	2209	2210	2211	2212	2213	2214	2215	2216	2217	2218	2219	2220	2221	2222	2223	2224	2225	2226	2227	2228	2229	2230	2231	2232	2233	2234	2235	2236	2237	2238	2239	2240	2241	2242	2243	2244	2245	2246	2247	2248	2249	2250	2251	2252	2253	2254	2255	2256	2257	2258	2259	2260	2261	2262	2263	2264	2265	2266	2267	2268	2269	2270	2271	2272	2273	2274	2275	2276	2277	2278	2279	2280	2281	2282	2283	2284	2285	2286	2287	2288	2289	2290	2291	2292	2293	2294	2295	2296	2297	2298	2299	2300	2301	2302	2303	2304	2305	2306	2307	2308	2309	2310	2311	2312	2313	2314	2315	2316	2317	2318</
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Figure 8

cNMP-BD

hGRF4
dGRF4
hEPAC
PRKAR1B
hPKGI1
m-EAG

hGRF4
dGRF4
hEPAC
PRKAR1B
hPKGII
m-EAG

FIGURE 9: SEQ ID NO: 1

GRF4-RA domain

Figure 9:

```

dgk-1a_ce_
GRF4
RaIGDS_h_
-----REDFE---IIRVFDGNS---YRSQIS-----RNIVVAKHVSQVQVRDAALR
--HHRILDFS---ATPDLPDQVLRVFKADQQS-----RYTMISKDTTAKVVIQAIR
SILVTSQDKAPSVISRVLKKNRDSAVASEYELVQLLPGERELTIPASANVFYAMDGASH
*          :  :          .: .          * : : . . . . * :
          :  :          .: .          * : : . . . . * :

dgk-1a_ce_
GRF4
RaIGDS_h_
RFHI--NDTPERYIT-QVVGEVEEILED-----PVP-----LRNVKRPEGKRAQIFIR
EFAV--TATPDQYSLC-EVSVTPGVIKQR-----RLP-----DQLSK--LADRIQLSGR
DFLLRHGEGPLLLHLASFVARLPQELLRVREEGAPFPGSRPQGGRLHGHCSSEEEAPLAYR
* : * : * : * : * : * : * : * : * : * :
* : * : * : * : * : * : * : * : * : * :

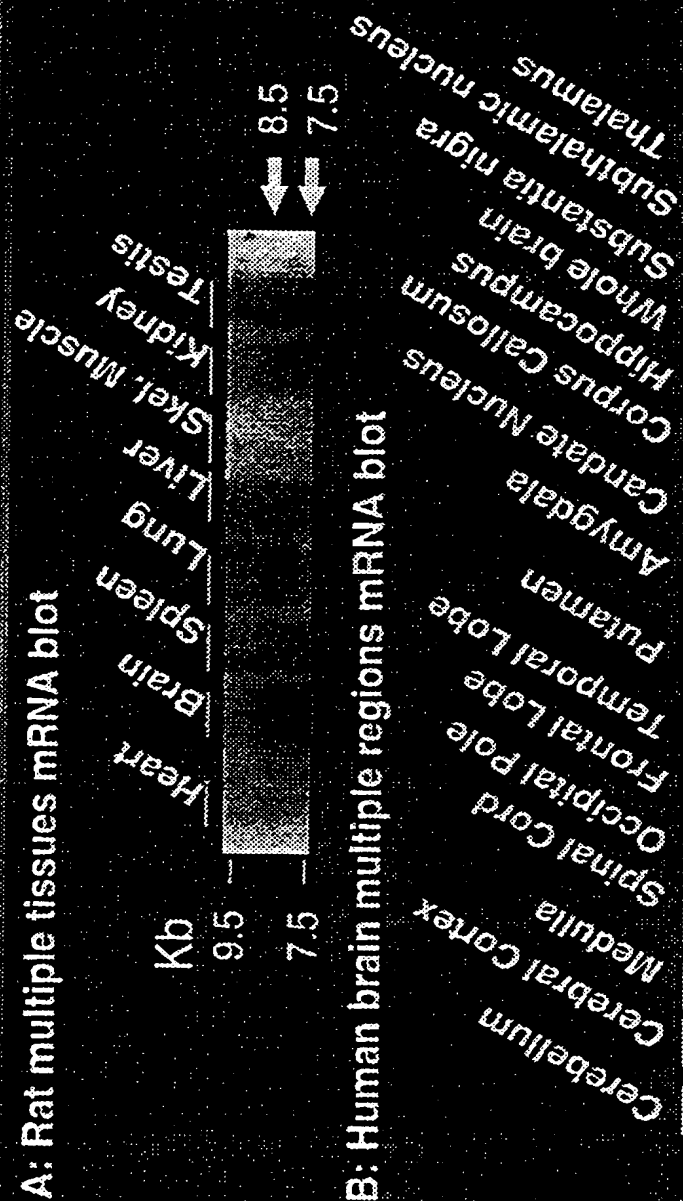
dgk-1a_ce_
GRF4
RaIGDS_h_
YYD-----
YYLKNNME-
SHGVHTRCG
:
```

T00240" 9224T660

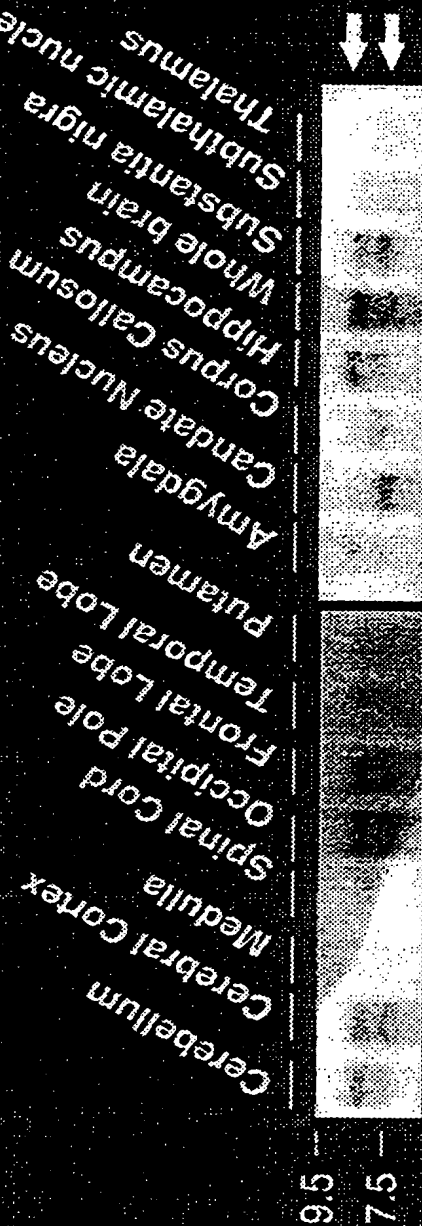
Figure 10

Tissue Distribution of GRF4

A: Rat multiple tissues mRNA blot



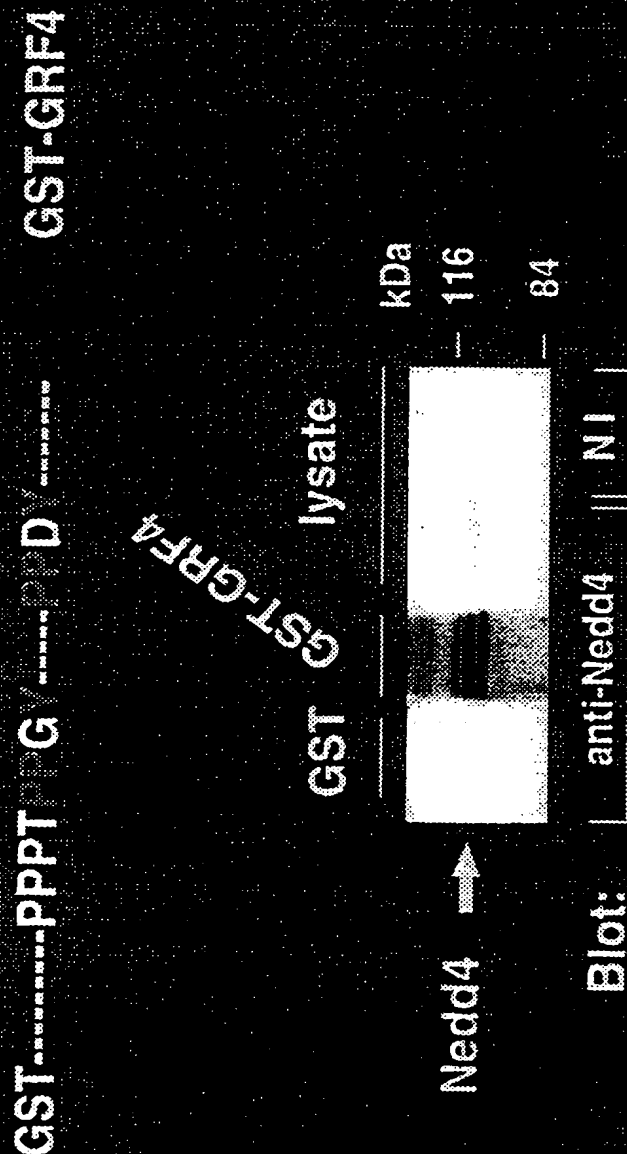
B: Human brain multiple regions mRNA blot



100220" 928TF660

Figure 11

GST-fusion protein of the C-terminal last 150 aa of
 GRF4 containing two PY motifs pulls down
 endogenous Nedd4 in Hek 293T cells



100220" 928T660

Figure 12

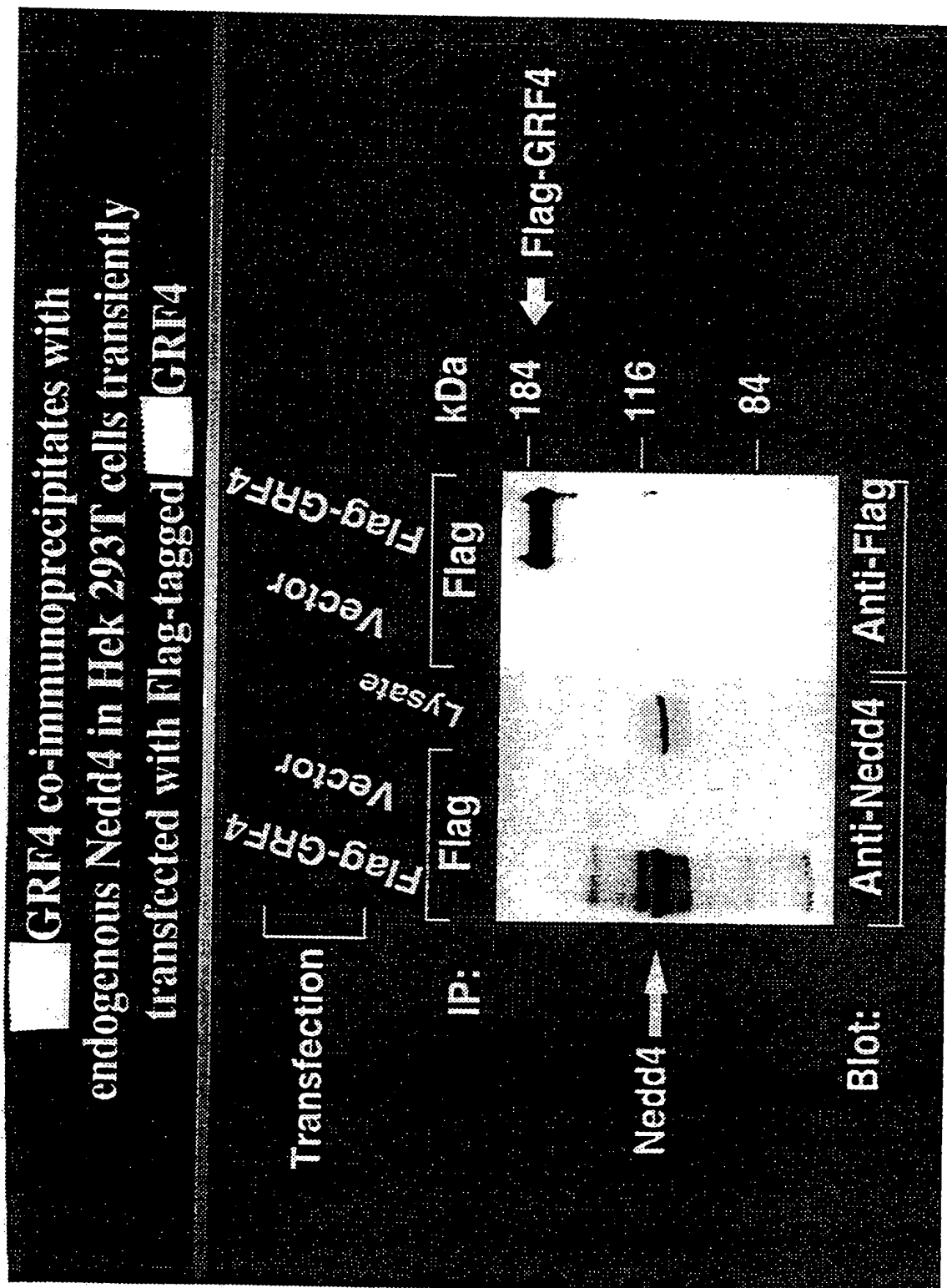


Fig.13

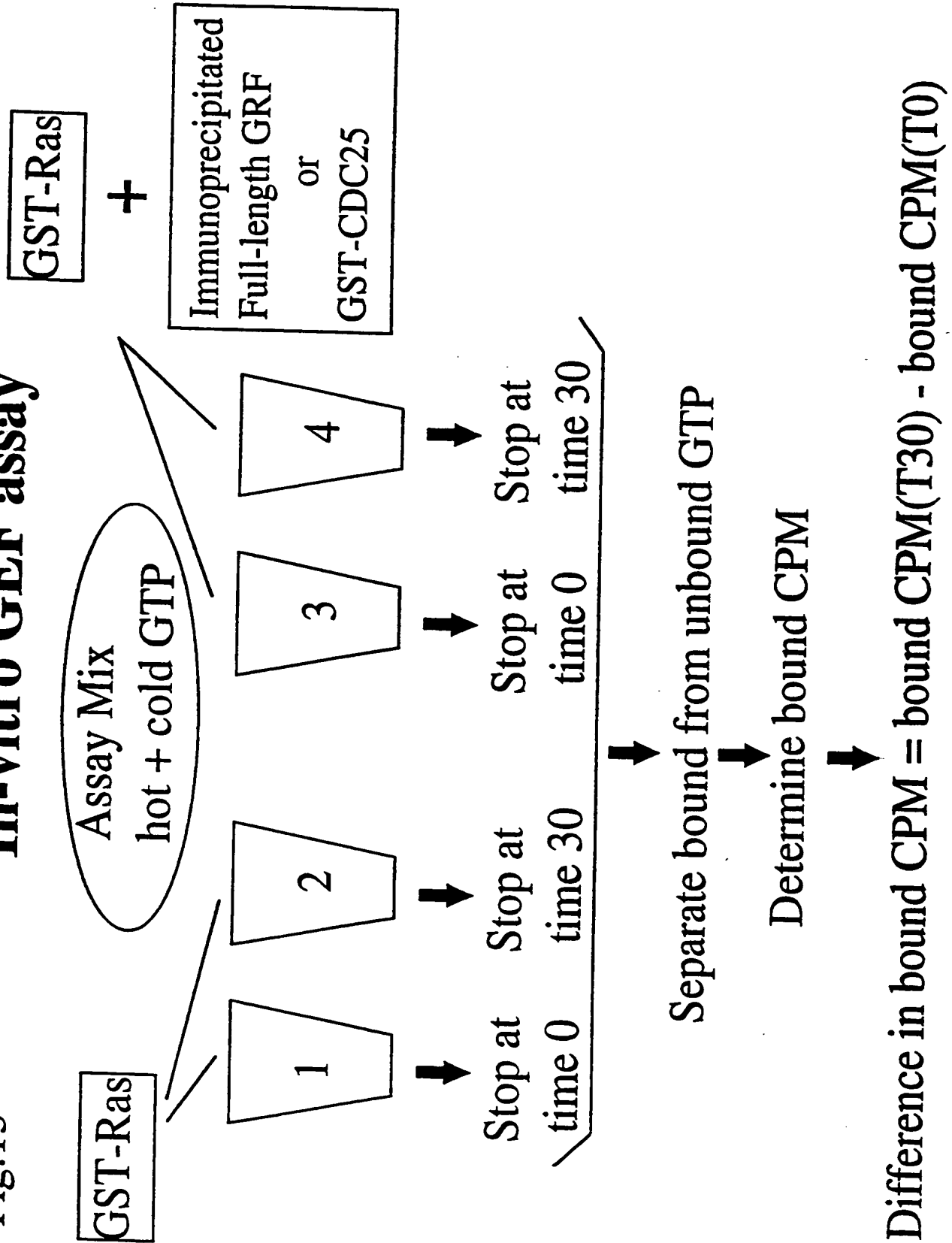
In-vitro GEF assay

FIG. 14

Fig.14 GRF4 is active on Ras : in-vitro GEF
assay using immunoprecipitated full-length
GRF4

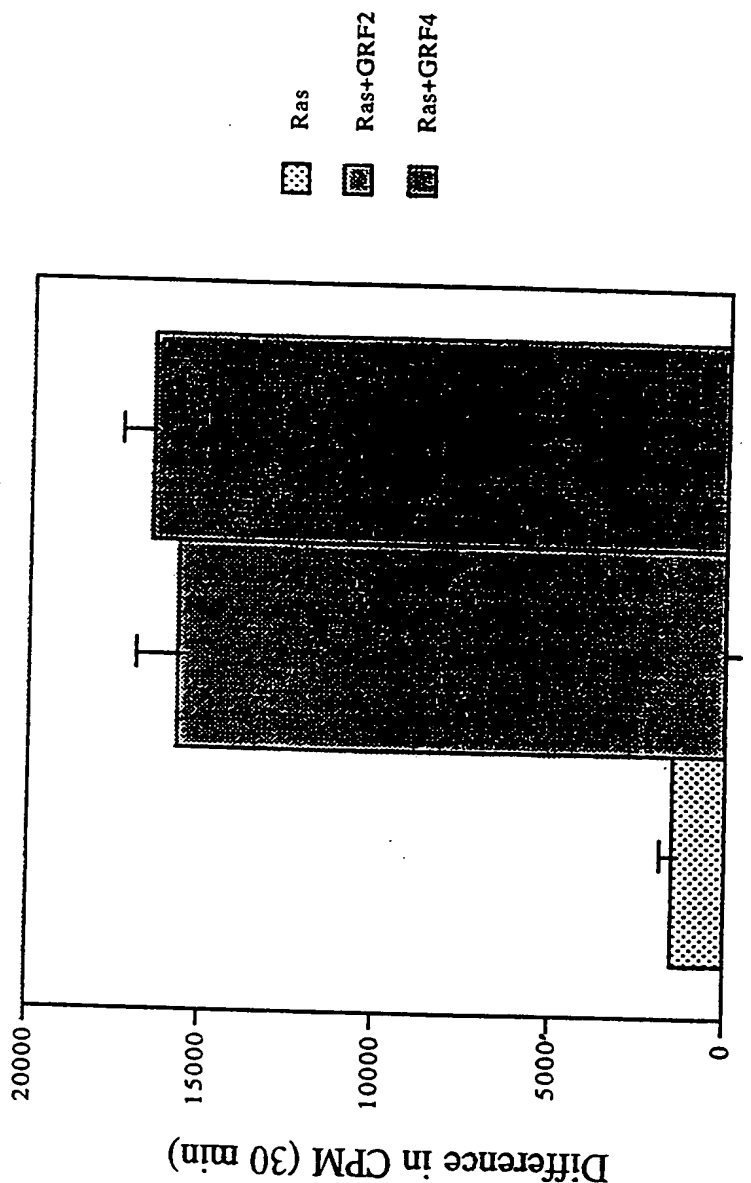


FIG. 15

Figure 15

GRF4 forms stable complex with Ras in-vitro:
it binds preferentially to nucleotide-free and
GTP-bound Ras

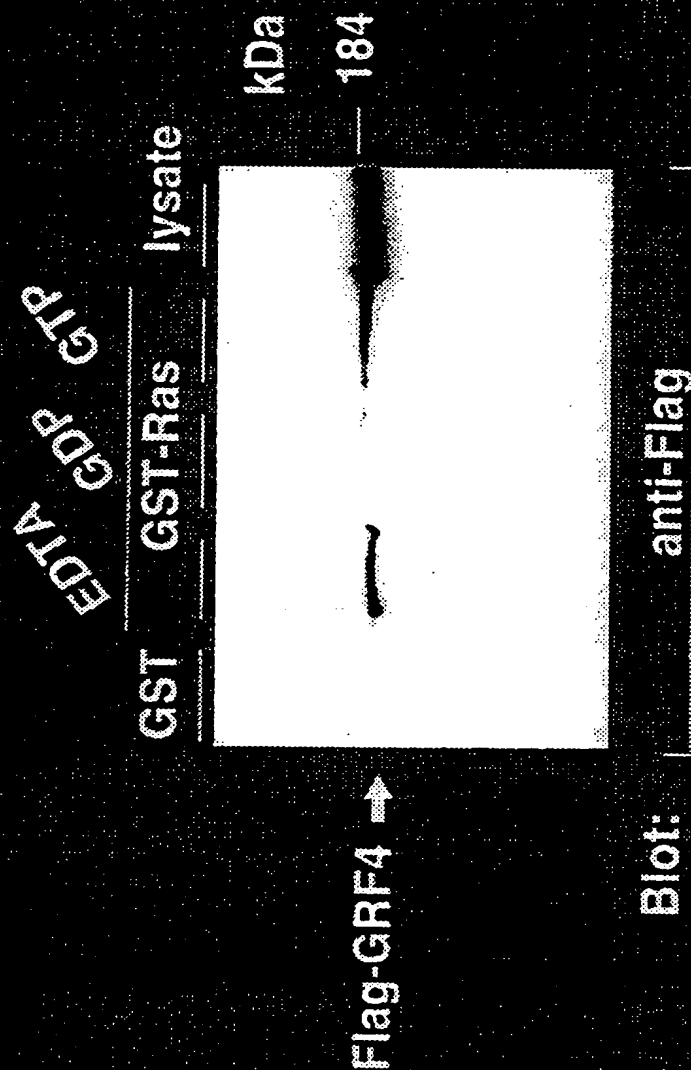


Figure 16

Transformation Assay:  GRF4 induces foci formation in Rat2 fibroblasts

Empty Vector  GRF4 RasV12



FIGURE 17

Figure 17

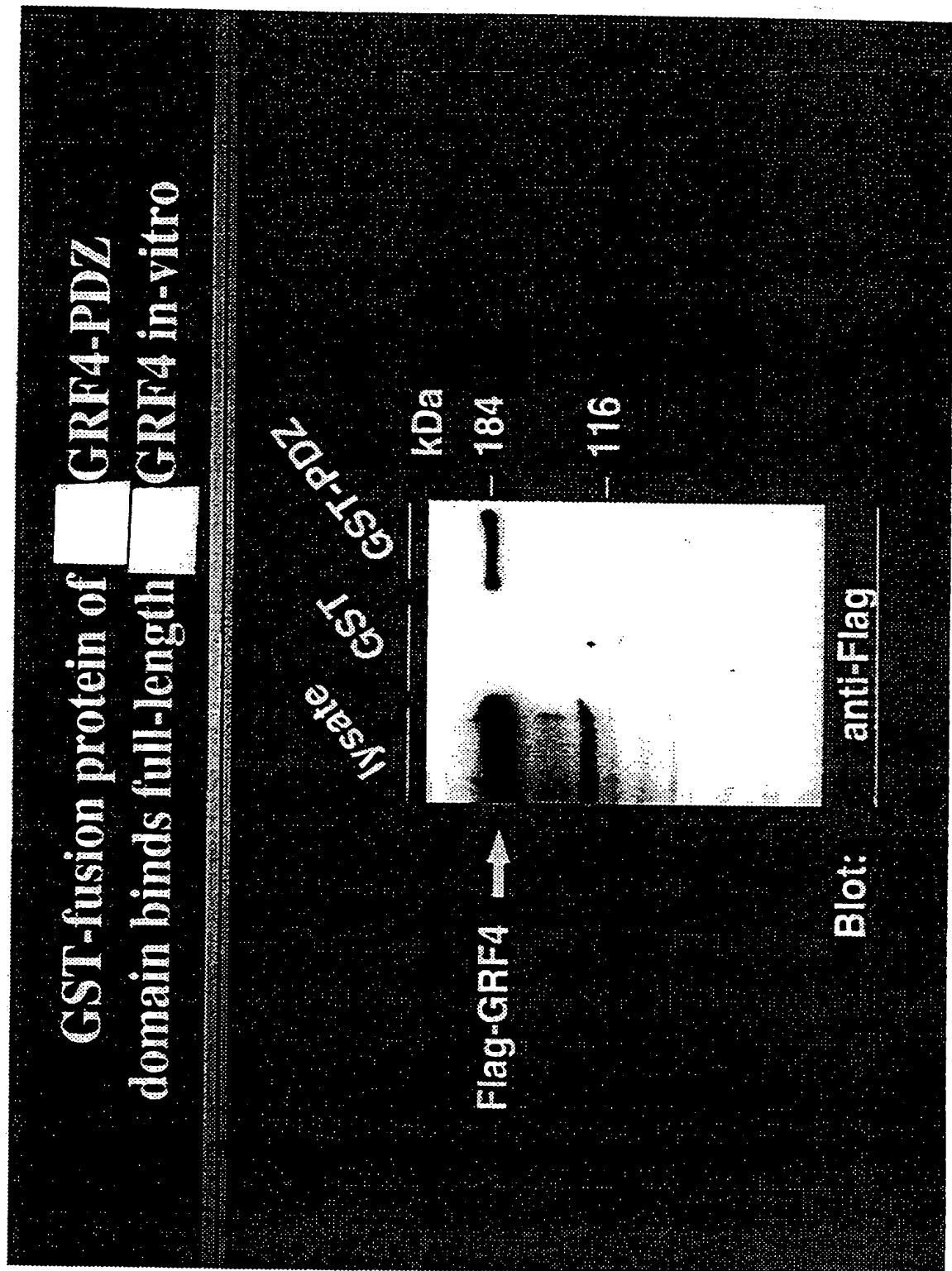
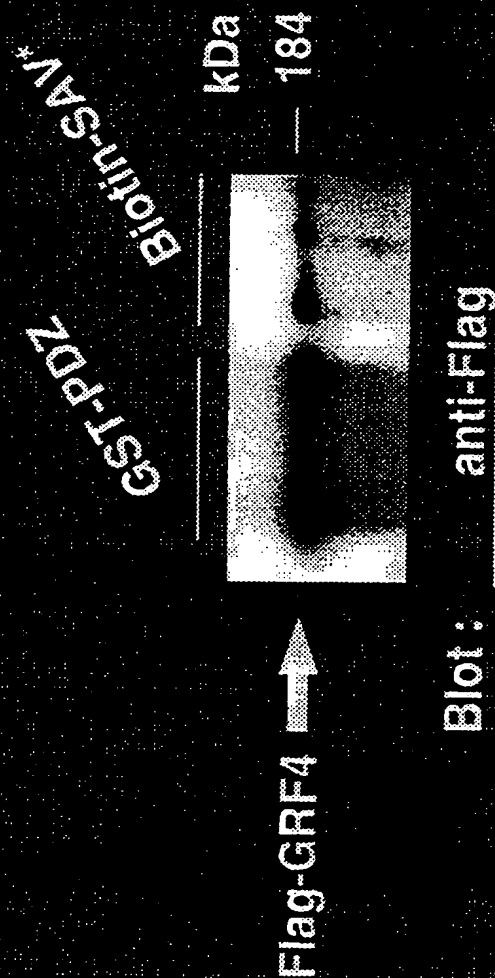


Figure 18

Biotinylated peptide of the last 15 aa sequence of
 [] GRF4 containing the [] PDZ-binding
 motif (SAV*) binds full-length [] GRF4 in-vitro



6568 bp

/translation="MKPLAIPANHGVMGQKEKHS LPADFTKLHLTDSLHPQVTHVSSS
HSGCSITSDSGSSSLSDIYQATESEAGDMDLSGLPETA VDSEDDDDDEEDIERASDPLM
SRDIVRDCLEKDPIDRTDDDIEQLLEFMHQLPAFANMTMSVRRELCAVMVFAVVERAG
TIVLNDGEELDSWSVILNGSVEVTPDGKAEILCMGNSFGVSPTMDKEYMKGMVMTKV
DDCQFVCIAQQDYCRILNQVEKNMQKVEEEGEIVMVKEHRELDRTGTRKGHIVIKGTS
ERLTMHLVEEHSVVDPTFIEDFLTYRTFLSSPMEVGKKLLEWFNDPSLRDKVTRVVL
LWVNNHFNDFEGDPAMTRFLEEFENNLEREKMGGLRLNIAACA AKARRLMTLTKPS
REAPLPFILLGGSEKGFIFVDSVDSGSKATEAGLKRGDQILEVNGQNFENIQLSKAM
EILRNNTLSITVKTNLFVKELLTRLSEEKRN GAPHLPKIGDIKKASRYSIPDLAVD
VEQVIGLEKVNKKS KANTVGGRNKLKKILDKTRISILPQKPYNDIGIGQSQDDSI VGL
RQTKHIPTALPVS GTLSSSNPDLLQSHHRLDFSATPDLPDQVLRVFKADQQSRYIMI
SKDTTAKEVVIAIREFAVATPDQYSLCEVSVTPGVIKQRRLPDQLSKLADRIQLS
GRYYLKNM ETETLCSDEDAQELLRESQISLLQLSTVEVATQLSMRNFLFRNIEPTE
YIDDLFLRSKTSCANLKRFEVINQETFWVASEILRETNQLKRMKIIKHFIKIALHC
RECKNFNSMFAISGLNLAPVARLRTTWEKLPNKYEKLFQDLQDLFDP SRNMAKYRNV
LNSQNLQPIIPLFPVIKKDLTFLHEGND SKVDGLVNFEKLRMAKEIRHVGRMASVN
MDPALMFRTRKKKWRSLGSLSQGSTNATVLDVAQTGGHKKRVRSSFLNAKKLYEDAQ
MARKVKQYLSNLELEMD EESLQTL SLQCEPATNTLPKNPGDKPKVKSETSPVAPRAGS
QQKAQSLPQPQQPPPAHKINQGLQVPAVSLYPSRKVPVKDLPPFGINSPQALKKIL
SLSEEGSLERHKQAEDTISNASSQLSSPPTSPQSSPRKGYTLAPSGTVDNFSDSGHS
EISSRSSIVSNSSFDSVPVSLHDERRQRHSVSIVETNLGMGRMERRTMIEPDQYSLGS
YAPMSEGRGLYATATVISSPSTEELS QDQGDRA SLDAADSGRGSWTS CSSGSHDNIQT
IQHORSWETL PFGHTHFDYSGDPAGLWASSSHMDQIMFSDHSTKYNRQNQSRESLEQA
QSRASWASSTGYWGEDSEGDTGTIKRRGGKDV SIEAESSLTSVTTEETKPVMPMAHI
AVASSTTKGLIARKEGRYREPPPTPPGYIGIPITDFEGHSH PARKPPDYNVALQSR
MVARSSDTAGPSSVQPHGHPTSSRPVNKPQWHKPNESD PRLAPYQSQGFSTEDEDE
QVSAV" [SEQ ID NO: 2]

63. .4562

BASE COUNT 1974 a 1400 c 1463 g 1731 t
ORIGIN

1 cttgccatcg tgagagattg gtacatgatg tgtaaattca gttcagcata tgtttcttca
61 ttatga aacc actagcaatc ccagctaacc atggagttat gggccagcag gagaaacact
121 cacttcctgc agatttcaca aaactgcac tctactgacag tctccacca caggtgaccc
181 acgtttcttc tagccattca ggatgtagta tctactagta ttctgggagc agcagttctt
241 ctgatatcta ccaggccaca gaaagcgagg ctggtgatat ggacctgagt ggggtgccag
301 aaacagcagt ggattccgaa gacgacgacg atgaagaaga cattgagaga gcatcagatc
361 ctctgatgag cagggacatt gtgagagact gcctagagaa ggaccaat gaccggacag
421 atgatgacat tgaacaactc ttggaattta tgcaccagtt gcctgcttt gccaatatga
481 caatgtcagt gaggcgagaa ctctgtgctg tgatggtgt cgcagtggtg gaaagagcag
541 ggaccatagt gtaaatgat ggtgaagagc tggactcctg gtcagtgatt ctcaatggat
601 ctgtggaagt gacttatcca gatggaaaag cagaaatact gtgcatggga aatagttttg
661 gtgtctctcc taccatggac aaagaataca tgaaggagat gatgagaaca aaggtggatg
721 actgccagtt tgtctgcata gccagcaag attactgccg tatttcaat caagtagaaa
781 agaacatgca aaaagttgaa gaggaaggag agattgttat ggtgaaagaa caccgagaac
841 ttgatcgaac tgaacaaga aaggacaca ttgtcatcaa ggttacctca gaaagggtta
901 caatgcattt ggtggaagag cattcagtag tagatccaac attcatagaa gactttctgt
961 tgacctatag gactttctt tctagcccaa tggaagtggg caaaaagtta ttggagtgtg
1021 ttaatgacc gagcctcagg gataaggta cacgggtagt attattgttg gtaataatc
1081 actcaatga cttgaagga gatcctgcaa tgactcgatt ttagaagaa ttgaaaaca
1141 atctggaaag agagaaaatg ggtggacacc taaggctgtt gaatatcgcg tgtctgtcta
1201 aagcaaaaag aagattgatg acgttaacaa aaccatcccg agaagctcct ttgccittta
1261 tcttacttg aggctctgag aagggtttg gaatctttgt tgacagtgt gattcaggta
1321 gcaaaagcaac tgaagcaggc ttgaaacggg ggatcagat attagaagta aatggccaaa
1381 acttgaaaa cattcagctg tcaaaagcta tggaaattct tagaataaac acacattat

(continued next page)

26/34

456| gaggcacaga cttttctgga agcagagcga gccacctgaa aggagagcac aagaagacgt
 462| cctgagcatt ggagccttgg aactcacatt ctgaggacgg tggaccagtt tgcctccttc
 468| ccigccttaa aagcagcatg gggtctcttc tccccctctt cmtccccct ttgcatgtga
 474| aatactgtga agaaattgcc ctggcacttt tcagactttg ttgcttgaaa tgcacagtgc
 480| agcaatcttc gagtcccccac ttttgctgcc tggcacatca cacagtatca ttccaaattc
 486| caagatcatc acaacaagat gattcactct ggctgcactt ctcaatgcct ggaaggattt
 492| ttttaactc tcttttaga tttcaatcca gtcttagcac ttgatctcat tgggataatg
 498| agaaaagcta gccattgaac tacttggggc ctttaaccca ccaaggaaga caaagaaaaa
 504| caatgaaatc ctttgagtac agtgcctgtc cactgttta caatgtctc ctttaaaaa
 510| aaaaaatgag tttaaagatt ttgttcagag agtaaatata tatccattta atgattacag
 516| tattattta aaccttaagt aggggttgcca gcctggttc tgaaaaacca aatatgcggg
 522| acagggtgtg gccacaccaa gaagacggga agacctggct tgtgacctg gcttcccatg
 528| tcttcttggt ctaccccgcg aagtgcctta tcttggaagt atgaaatgtt agccaattaa
 534| taccaagaca cctcatctgc tcttcccca gtggatgggg ttctctgta aaactgtttg
 540| cacatggcca ggggagggaa ctaggacct tgtgtctgt ctgagcctta tggagcgagg
 546| acggtgtcat tggcggatgt gtctgtctc attgagatgg atggcaaac ccatmttaa
 552| gttatattc ttgtatttt gtttaattag aggtgtaggt ttgttttt gttttgtt
 558| tttttaag agaaacattt ataactggat agcattgcag tgaagcagc ttgggatgtt
 564| ggagctaatt ccagctgttt atactgtct tcaagacag cctcccttta ttgaattggc
 570| attaggaat aaacaagcct taaacgtga taaaagatca aaaacctggt tagacatgcc
 576| agccttgca aggcagggtta gtcaccaaag actaacctcc aagtggcttt atggacgtg
 582| catatagaga aggcctaagt gtagcaacca tctgtcaca gctgtattta accctataat
 588| gactgaaatg accctccac tctattttg tttgttttg cacagactcc gaaaaagtga
 594| aggtgccaa tctgagtagt actcaaatgt gaggaactgc tggcttgga ttttttcc
 600| attaaattca gctgacata ttgatcagta gataaacgta aatagcttca aattttaaaa
 606| gtggaattgc agtgttttt cactgtatca aacaatgtca gtgctttatt taataattct
 612| ctctgtatc atggcattg tctacttct tattacattg tcaattatgc atttgaatt
 618| ttacatgtaa tatgcattat ttgccagttt tattatata gctatggacc tcatgtgcat
 624| atagaaagac agaaatctag ctctaccaca agttgcacaa atgttatcta agcattaagt
 630| aatttagaa cataggactg ctaatctcag ttgctctgt gatgtcaagt gcagaatga
 636| caattaactg gtgatttct catactttg atactactg tacctgtatg tcttttagaa
 642| agacattggt ggagtctgta tccctttgt attttaata caataattgt acataattgt
 648| tatattttg tgaagatgg tagaaatgta ctatgttat gcttctacat ccagtttgta
 654| caagctggaa aataaataaa tataacat [SEQ ID NO: 1]

801 b.p.

1/1
 ACT AAA GGG AAC AAA AGC TGG AGC TCC ACC GCG GTG GCG GCC GCT CTA GAA CTA GTG GAT
 T K G N K S W S S T A V A A A L E L V D
 L K G T K A G A P P R W R P L N W I
 R E Q K L E L H R G G G R S R T S G S

31/11
 61/21
 CCC CCG GGC TGC AGG AAT TGA AGC GGT GGG AAG GAT GTC TCC GCT GAG GCA GAG AGC AGC
 P P G C R N S S G G K D V S A E A E S S
 P R A A G I Q A V G R M S P L R Q R A A
 P G L Q E F K R W E G C L R G R E Q Q

91/31
 121/41
 AGC ATG GTG CCC GTG ACT ACA GAG GAA GCC AAA CCT GTC CCT ATG CCT GCC CAC ATA GCT
 S M V P V T T E E A K P V P M P A H I A
 A W C P L Q R K P N L S L C L P T L
 H G A R D Y R G S Q T C P Y A C P H S C

151/51
 181/61
 GTG ACG CCG AGC ACT ACC AAG GGA CTC ATC GCA CGG AAG GAA GGC AGG TAC CCG GAG CCG
 V T P S T T K G L I A R K E G R Y R E P
 R R A L P R D S S H G R K A G T G S R
 D A E H Y Q G T H R T E G R Q V P G A A

211/71
 241/81
 CCT CCC ACA CCT CCA GGC TAC GTG GGC ATC CCC ATT GCC GAT TTC CCA GAA GGG CCT TGC
 P P T P P G Y V G I P I A D F P E G P C
 L P H L Q A T W A S P L P I S Q K G L A
 S H T S R L R G H P H C R F P R R A L P

271/91
 301/101
 CAC CCG GCC AGG AAG CCC CCG GAT TAC AAC GTG GCC CTG CAG CCG TCC CGC ATG GTG GCA
 H P A R K P P D Y N V A L Q R S R M V A
 T R P G S P R I T T W P C S G P A W W H
 P G Q E A P G L Q R G P A A V P H G G T

331/111
 361/121
 CCG CCC ACT GAG GCC CCG GCA CCG GGC CAG ACG CCG CCT GCA GCC GCA GCC AGC CCG CCG
 R P T E A P A P G Q T P P A A A A S R P
 G P L R P R A R R R L Q P Q P A G R
 A H G P G T G P D A A C S R S Q P A G

391/131
 421/141
 GGC AGC AAG CCA CAG TGG CAC AAG CCC AGC GAC GCA GAC CCA CGC CTC GCG CCC TTC CAG
 G S K P Q W H K P S D A D P R L A P F Q
 A A S H S G T S P A T Q T H A S R P S S
 Q Q A T V A Q A Q R R R P T P R A L P A

451/151
 481/161
 CCG CAG GCT TCG CAG GAG CCG AGG AGG ACG AAG ATG AAC AAG TGT CTG CTG TTT GAG GCG
 P Q A S Q E R R T K M N K C L L F E A
 R R L R R S G G G R R T S V C C L R R
 A G F A G A E E D E D E Q V S A V G A

511/171
 541/181
 CAG GCT CCT TGA TCC ACA GTG AGC CAC CCA AAG GAG AGC ACA AGA AGA CCT CCC AAG CCT
 Q A P S T V S H P K E S T R R R P K P
 R L L D P Q A T Q R R A Q E D V P S L
 G S L I H S E P P K G E H K K T S Q A L

571/191
 601/201
 TGG AGC CTT GGC ACG CAC ATC TGA GGA TGG TGG ACC AGT TTG CCT CCT TCC CTG CCT TAA
 W S L G T H I G W W T S L P P S L P
 G A L A R T S E D G G P V C L L P C L K
 E P W H A H L R M V D Q F A S F P A L K

631/211
 661/221
 AGC AGC ATG GGG CTT CTT CTC CCC TTC TTC CTT TCC CCT TTG CAT GTG AAA TAC TGT GAA
 S S M G L L L P F F L S P L H V K Y C E
 A A W G F F S P S S F P L C M N T V K
 Q H G A S S P L L P F P F A C E I L R

691/231

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721/241

751/251

GAA ATT GCC CTG GCA CTT TGC AGA CTT GTT GCT TGA AAT GCA CAG CCC AGC AGC CCC TGA
 E I A L A L C R L V A * N A Q P S S P *
 K L P W H F A D L L L E M H S P A A P E
 N C P G T L Q T C C L K C T A Q Q P L S

781/261

GCT GCT GCC TGC CAC GTC ACG [SEQ ID NO: 3]
 A A A C H V T [SEQ ID NO: 4]
 L L P A T S [SEQ ID NO: 5]
 C C L P R H [SEQ ID NO: 6]

T00240" 928T660

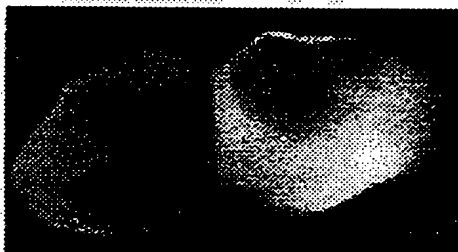
The PDZ domain (but not the C terminal SxV motif)
is required for plasma membrane localization of
GRF4

GRF4 Localization

WT



-PDZ



-SaV

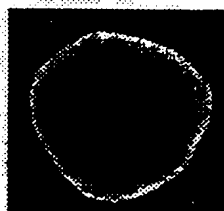


Figure 20

Figure 21

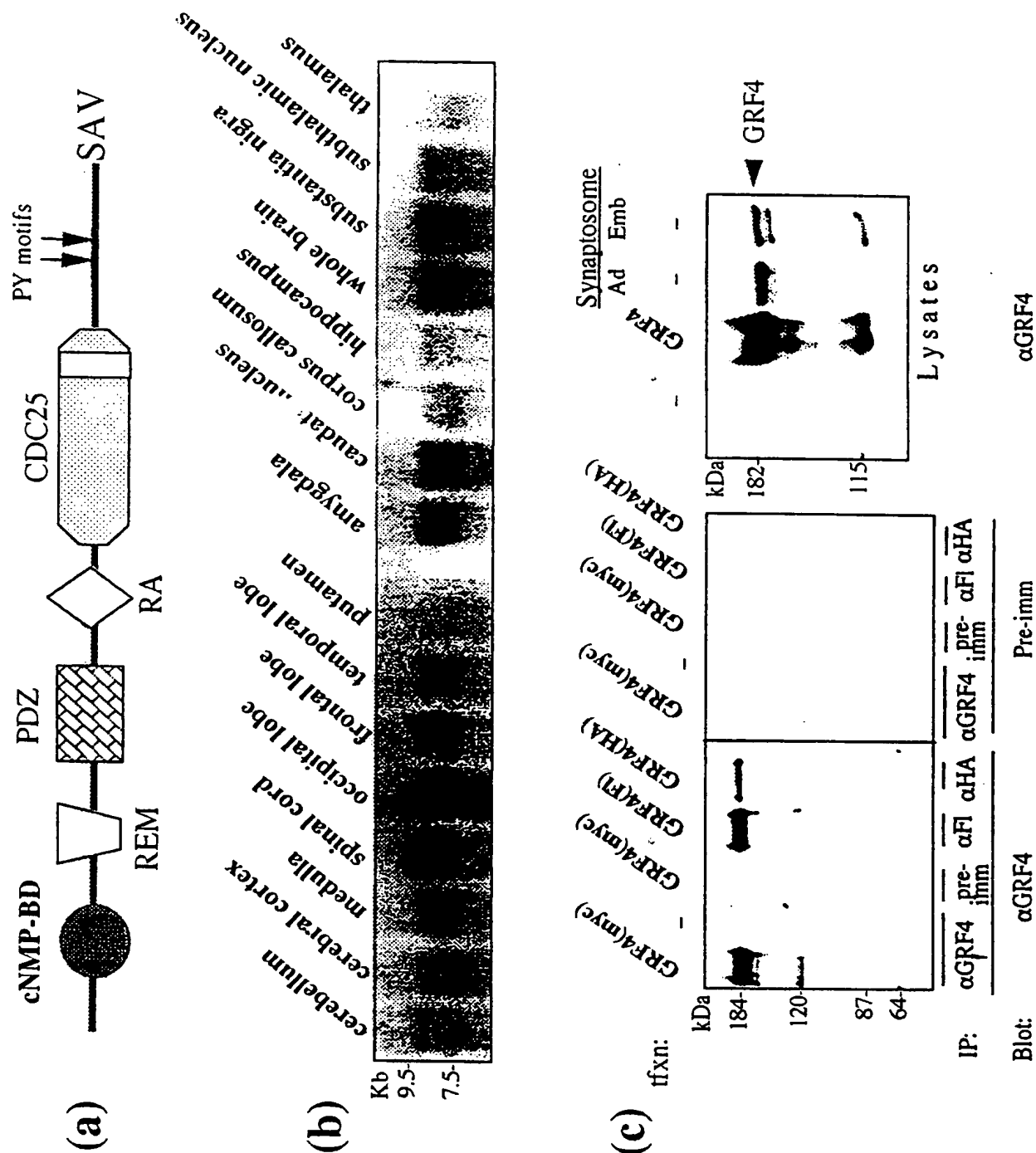


Figure 22

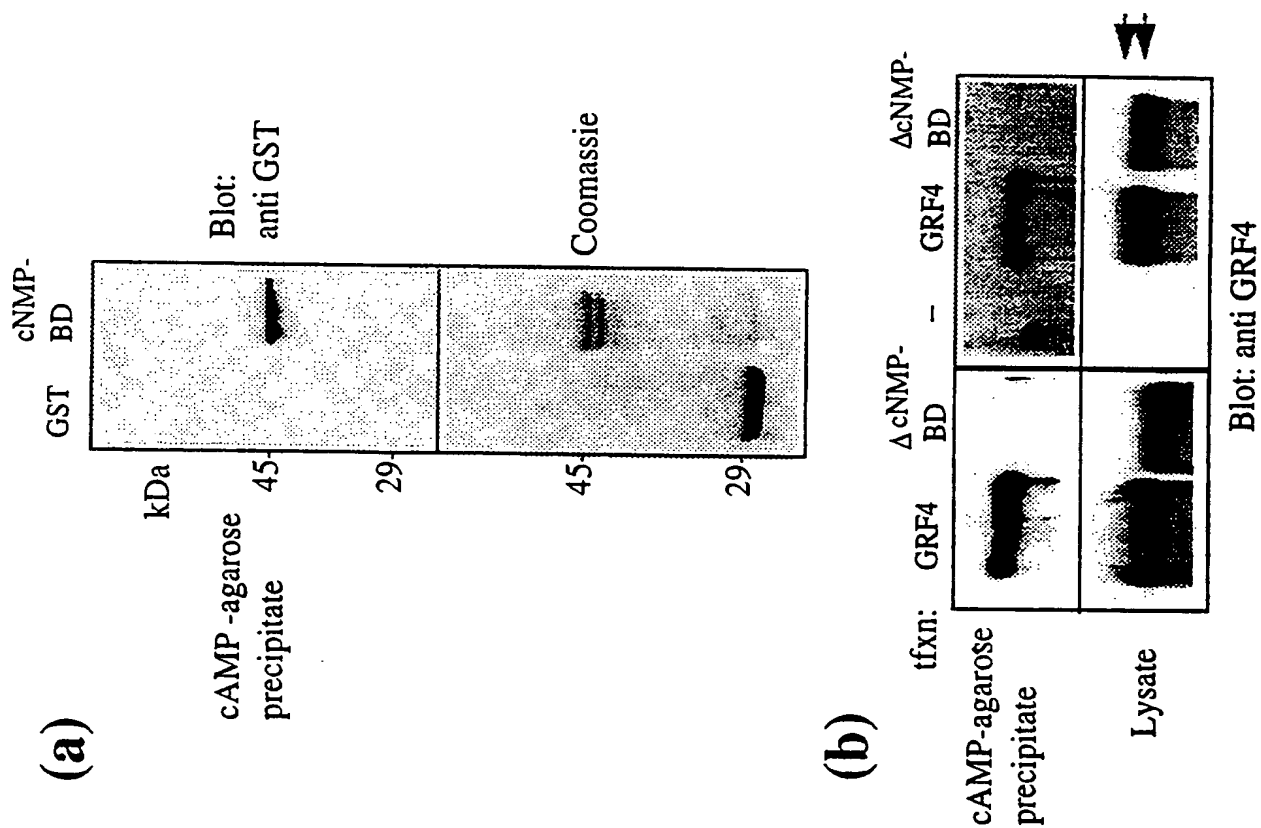
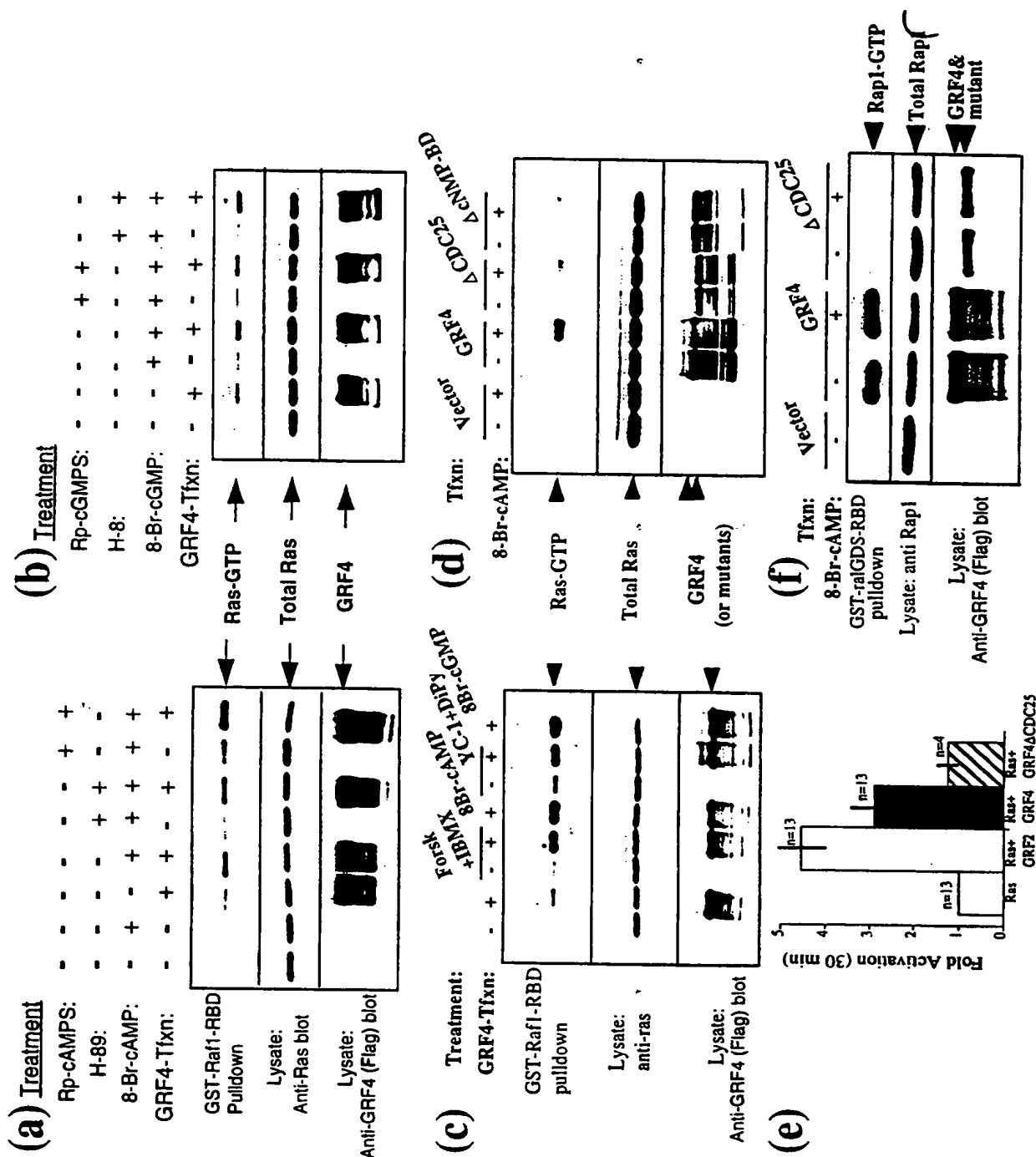


Figure 23



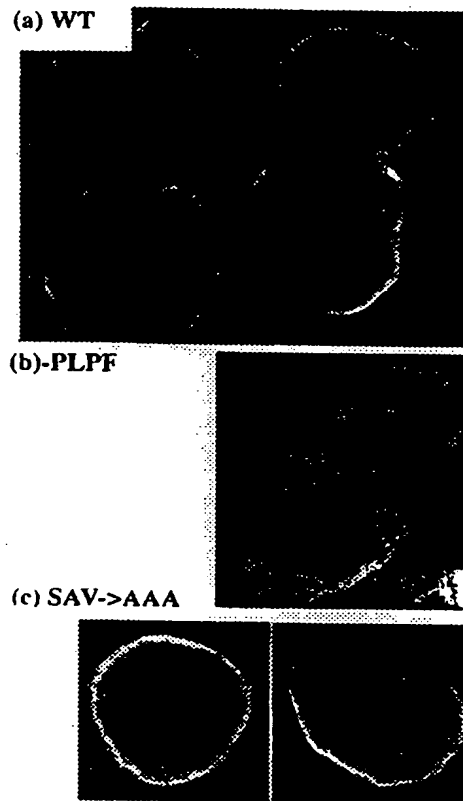


Figure 24